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*Publication date:*  
2016

*Document Version*  
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

*Citation (APA):*  
Kristensen, K., Urquhart, A., Thormann, E., & Andresen, T. L. (2016). *Binding of human serum albumin to liposomes studied by fluorescence correlation spectroscopy*. Poster session presented at The 43rd Annual Meeting & Exposition of the Controlled Release Society, Seattle, WA, United States.

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# Binding of human serum albumin to liposomes studied by fluorescence correlation spectroscopy

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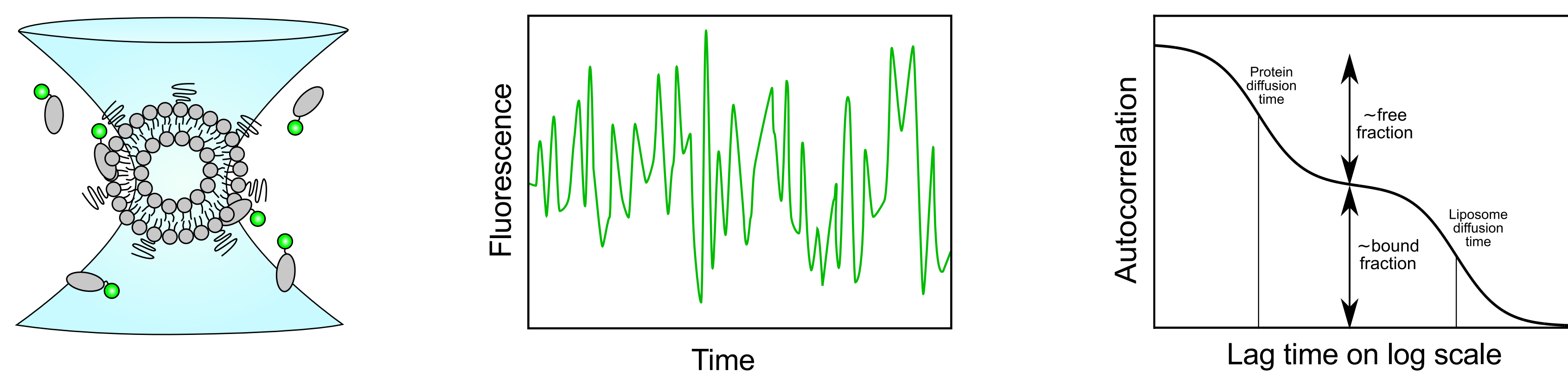
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## Introduction

Liposomal drug carriers are often administered into the blood. Once in circulation, the liposomes are covered with a "protein corona", conferring a new biological identity to the liposomes.<sup>1</sup> For example, the protein corona may both impact the circulation properties and targeting capabilities of liposomes.<sup>2</sup> Accordingly, to rationally design novel liposomal drug delivery systems, deep knowledge about the protein corona is required. So far, there is a lack of knowledge about the role of human serum albumin (HSA)—the most abundant protein in human blood plasma—in the corona. This is, in part, due to a lack of knowledge about the affinity of HSA for binding to standard liposomes and the dynamics of the binding process. Therefore, we have used fluorescence correlation spectroscopy to study the binding of HSA to different types of PEGylated fluid-phase liposomes (consisting of DOPC and DOPE-PEG2k) and PEGylated gel-phase liposomes (consisting of DSPC and DSPE-PEG2k) with various PEG chain surface densities.

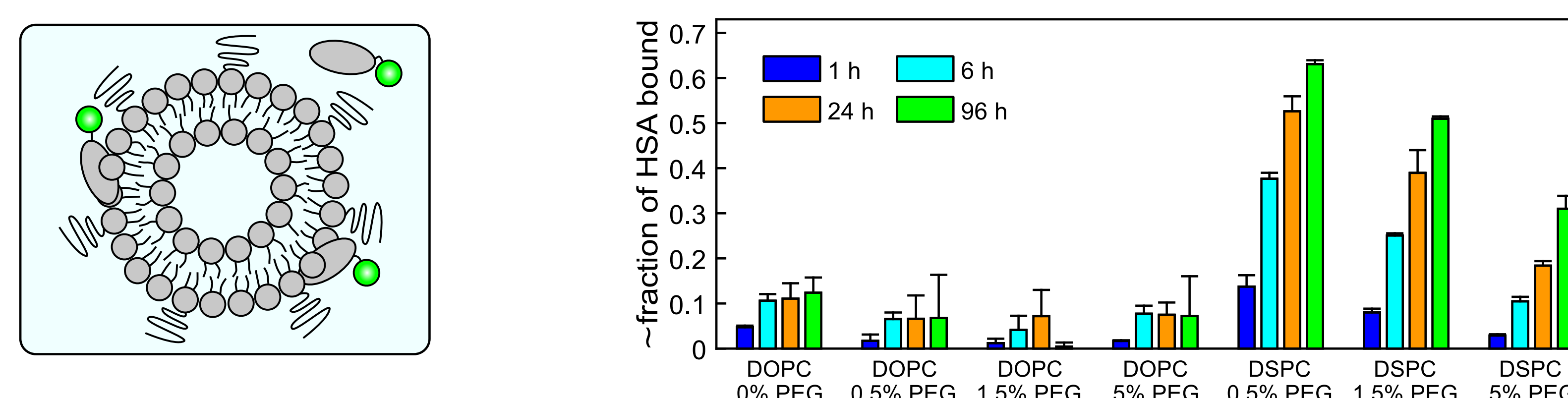
## Principle

Fluorescence correlation spectroscopy (FCS) measures the fluorescence emission intensity from fluorescent particles diffusing across a tiny focal detection volume. Autocorrelation analysis of the intensity time trace gives information about the concentration and diffusion properties of the particles.<sup>3</sup> Liposome-bound fluorescently labeled proteins will make a different diffusion contribution to the autocorrelation curve than free fluorescently labeled proteins. By this principle, FCS can provide detailed information about the liposome binding affinity and dynamics of proteins.<sup>4</sup>



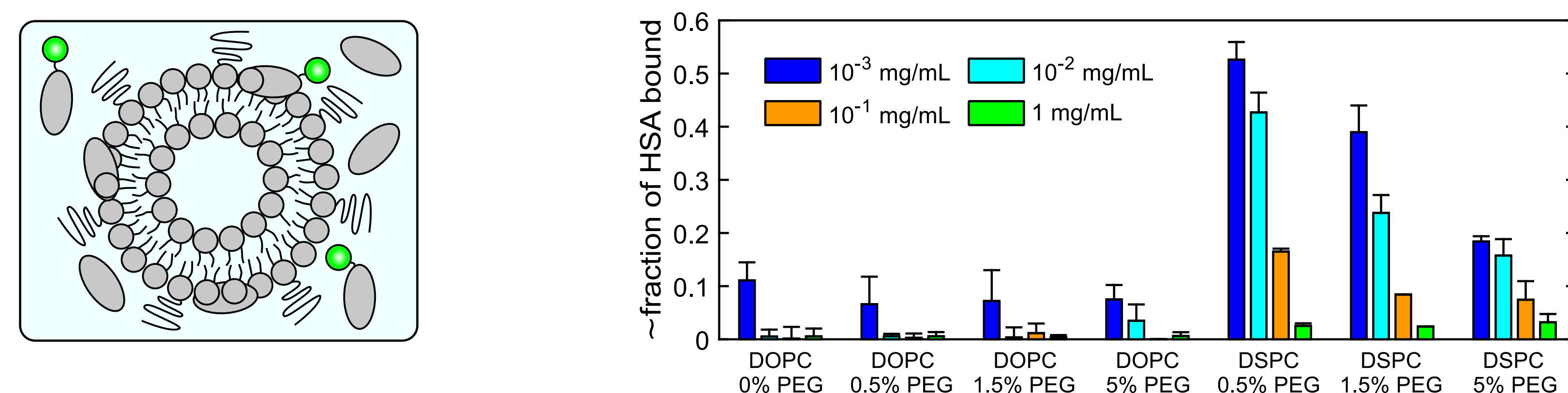
## Binding kinetics

We first considered the binding of  $1 \times 10^{-3}$  mg/mL labeled HSA to different DOPC-based and DSPC-based PEGylated liposomes (10 mM lipid concentration) in samples incubated for different times at 37 °C. There was only little binding of HSA to the DOPC-based PEGylated liposomes. In contrast, there was appreciable binding of HSA to the DSPC-based PEGylated liposomes, although the binding kinetics were very slow. Of relevance, HSA binding to the DSPC-based PEGylated liposomes decreased as the PEG chain surface density increased.



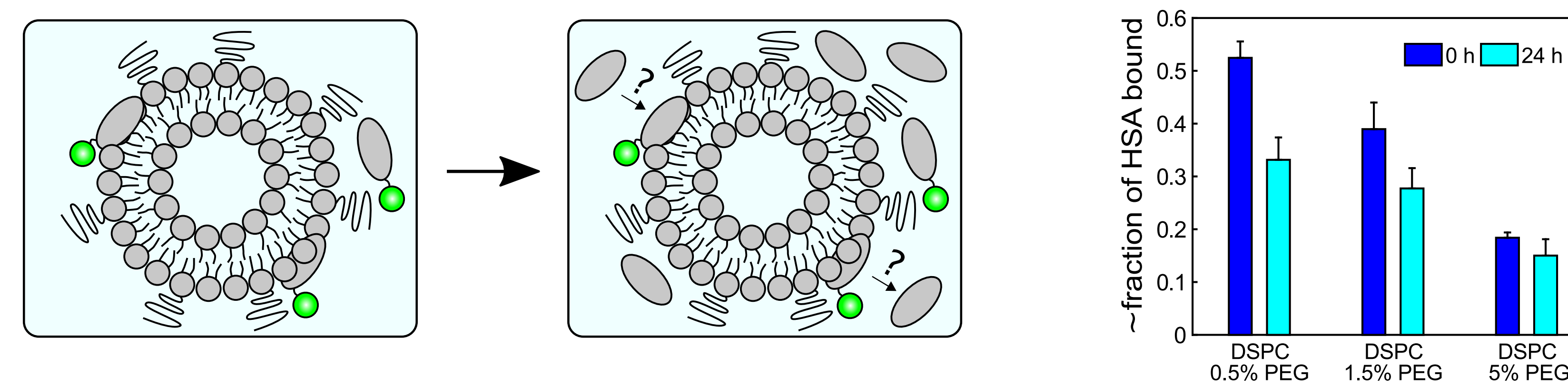
## Surface saturation

We next considered the binding of  $1 \times 10^{-3}$  mg/mL labeled HSA to different DOPC-based and DSPC-based PEGylated liposomes (10 mM lipid concentration) in samples with varying concentrations of unlabeled HSA between 0-1 mg/mL incubated for 24 h at 37 °C. There was no significant binding of HSA to the DOPC-based PEGylated liposomes. In contrast, there was considerable binding of HSA to the DSPC-based PEGylated liposomes, albeit the liposomes became saturated at an HSA concentration of 1 mg/mL, indicating that maximally 5 HSA molecules could bind per liposome.



## Exchange kinetics

We finally considered an experiment in which  $1 \times 10^{-3}$  mg/mL labeled HSA was incubated with DSPC-based PEGylated liposomes (10 mM lipid concentration) for 24 h at 37 °C. Then, 1 mg/mL unlabeled HSA was added and the samples were incubated for another 24 h at 37 °C to check whether the unlabeled HSA would cause the labeled HSA to dissociate from the liposomes. Similar binding levels were measured at the point of and 24 h after addition of unlabeled HSA, indicating that HSA generally bound tightly to the DSPC-based PEGylated liposomes.



## Conclusions

We detected no significant binding of HSA to the DOPC-based PEGylated liposomes. In contrast, we found that HSA bound tightly to the DSPC-based PEGylated liposomes, albeit these liposomes only presented a limited number of HSA binding sites. Possibly, these binding sites represent membrane packing defects as such defects are found in gel-phase membranes causing exposure of hydrophobic domains. Overall, our results suggest that the investigated liposomes cannot be covered with a layer of HSA, not even a loosely bound layer.

## References

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## Acknowledgements

Financial support for this work was kindly provided by the Lundbeck Foundation Research Initiative on Brain Barriers and Drug Delivery.



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